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DEVELOPMENT OF A THERAPEUTIC AGENT FOR WOUND-HEALING ENHANCEMENT

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SCIENTIFIC PROGRESS DURING THE LAST QUARTER

PEPTIDE SYNTHESIS

During this quarter we synthesized and purified three peptides, one from PDGF A-chain sequence and two from PDGF B-chain sequence. These peptides are listed below.

PDGF A (101-125)	Peptide #3, Table 9, p. 23 in the original proposal.
PDGF B (104-116)	Peptide #7, Table 4, p. 18 in the original proposal.
PDGF B (115-128)	Peptide #8, Table 4, p. 18 in the original proposal.

BIOLOGICAL ASSAYS

During this quarter we conducted cell binding experiments and assays for mitogenesis. These assays are described below.

CELL BINDING ASSAYS (COMPETITION FOR RECEPTOR BINDING)

Competitive receptor binding assays were performed on four peptides using NIH 3T3 cells. The results are shown in Table 1 and Figures 1 through 5. As seen in Table 1, none of the peptides tested so far showed any binding. We tested two peptides at three doses and two at four doses. In the future, we will test peptides initially at three doses. Active peptides will be tested at four or more doses.

Statement A per telecon
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Table 1
RESULTS OF COMPETITIVE BINDING ASSAYS

Peptide	Concentration (ng or μ g well)	CPM	Average \pm SD	% Maximum Binding	% Inhibition
Control (Empty wells, no cells)		280.746 314.922 282.753 287.973	290 \pm 11		
125 I PDGF B	5 ng	25291.9 26844.3 26228.4	26,121 \pm 781	100	0
PDGF B	100 ng	7605.88 7693.76 6841.80	7,381 \pm 469	28	72
	50 ng	9548.28 9074.44 8906.09	9,176 \pm 333	35	65
	25 ng	11,152.7 11,619.7 10,649.4	11,141 \pm 486	43	57
	12.5 ng	13,065.2 13,860.2	13,462 \pm 562	51	49
	6.25 ng	15,780.8 17,614.3	16,698 \pm 1296	64	36
PDGF A (12-28)	500 μ g	23,587.2 23,163.3 25,874.6	24,208 \pm 145	93	7
	250 μ g	26,241.3 25,861.5 23,451.4	25,184 \pm 1513	96	4
	125 μ g	23,002.4 24,337.9 24,966.6	24,102 \pm 1003	92	8
PDGF B (22-36)	500 μ g	23,036.7 23,927.8 22,716.3	23,225 \pm 625	89	11
	100 μ g	23,637.7 21,963.2 23,276.3	22,959 \pm 881	88	12
	50 μ g	24,241.9 24,307.9 24,731.0	24,427 \pm 265	94	6
	10 μ g	25,460.8 27,353.8 26,969.7	26,928 \pm 448	103	-3

Table 1 (concluded)

Peptide	Concentration (ng or µg well)	CPM	Average ± SD	% Maximum Binding	% Inhibition
PDGF B (101-117)	500 µg	24,978.2	24,046.3	92	8
		24,618.9			
		22,541.8			
	250 µg	24,000.0	23,501 ± 535	90	10
		23,567.7			
		22,936.9			
	125 µg	23,997.8	23,602 ± 902	90	10
		24,238.9			
		22,570.4			
PDGF B (104-116)	500 µg	26,246.9	26,468 ± 423	101	-1
		26,200.9			
		26,955.5			
	100 µg	25,191.7	26,678 ± 1915	102	-2
		26,001.8			
		28,839.2			
	50 µg	26,491.3	27,164 ± 904	104	-4
		28,192.3			
		26,807.4			
	10 µg	26,560.7	26,585 ± 438	102	-2
		26,159.8			
		27,034.1			

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF A (12-28)**

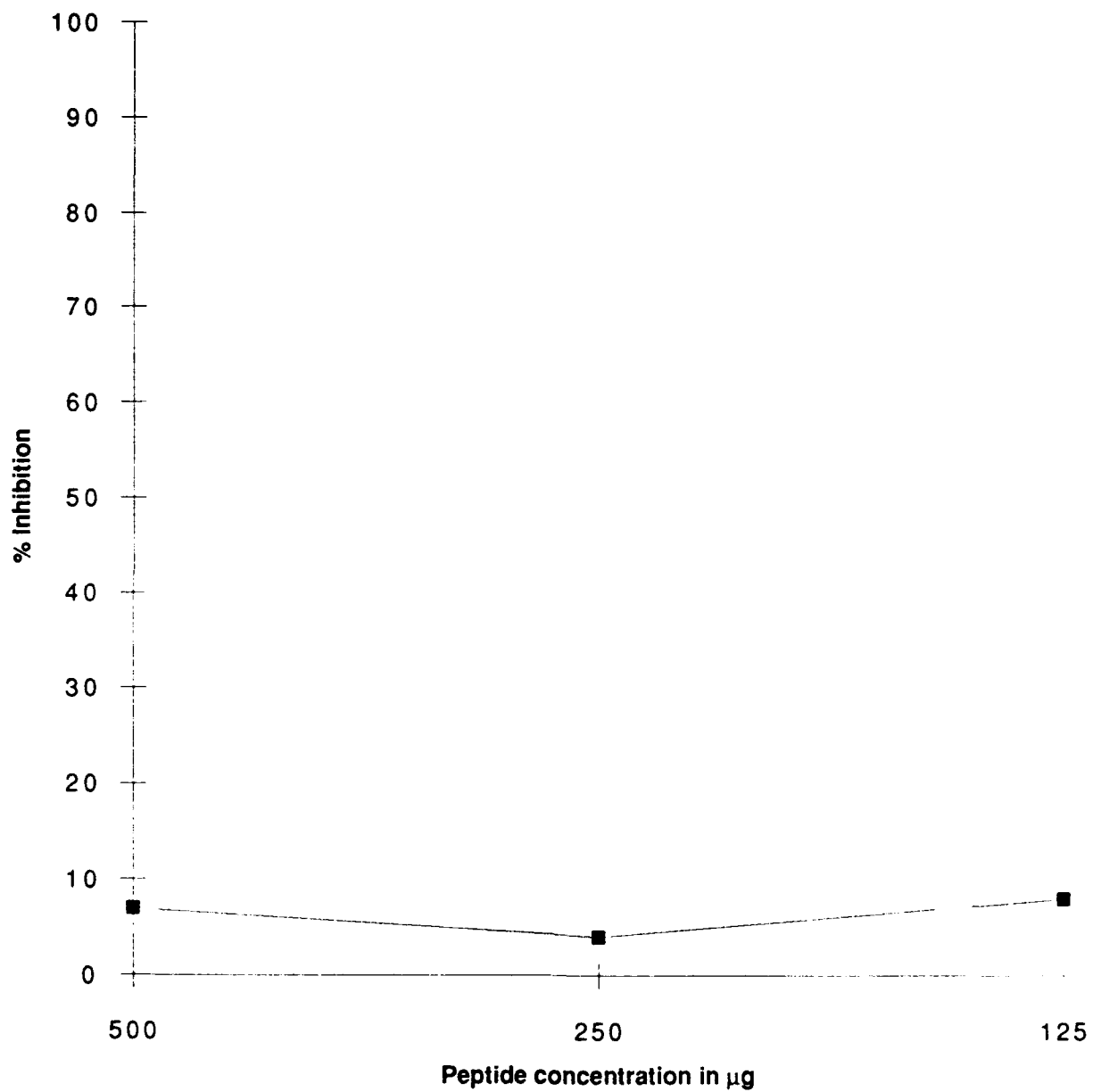


Figure 1

**Competitive inhibition for Receptor Binding
on 3T3 cells by PDGF B (22-36)**

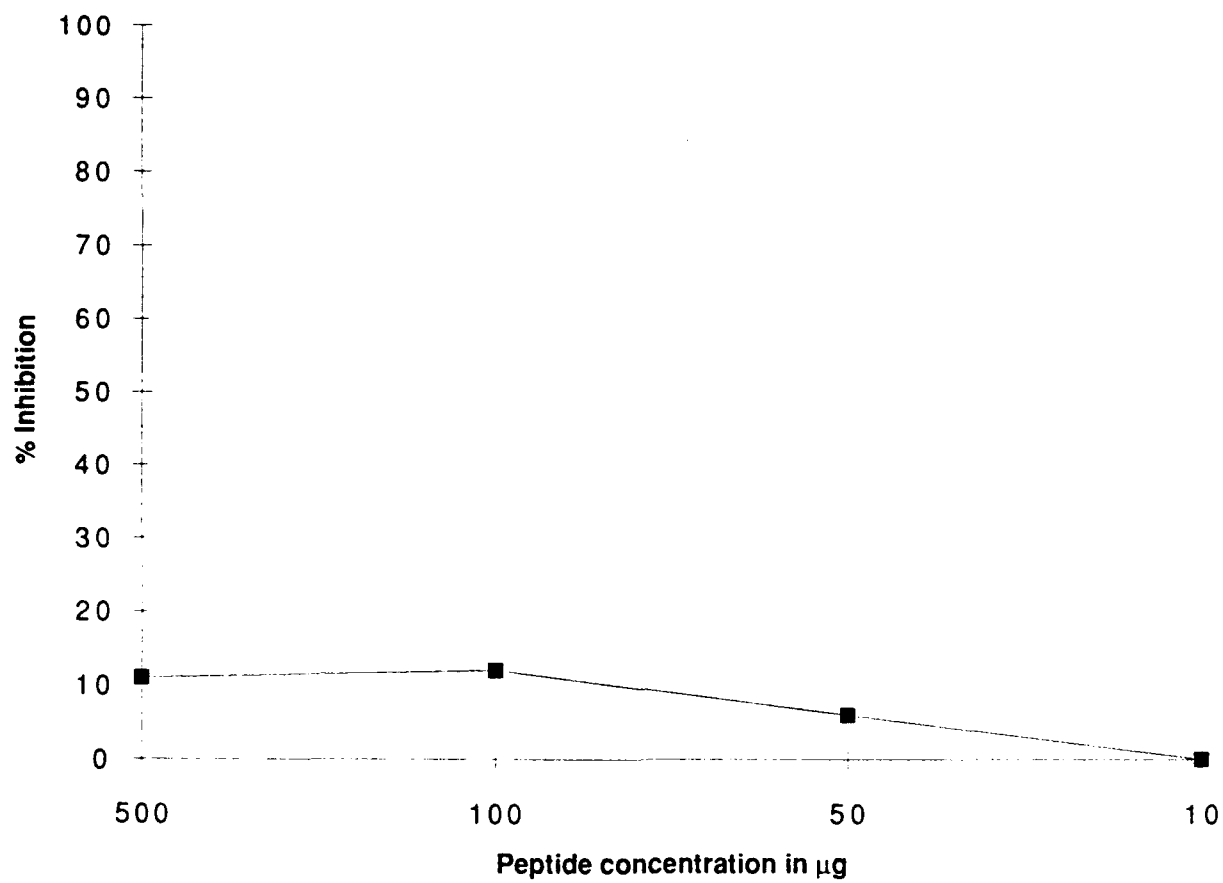


Figure 2

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF B (101-117)**

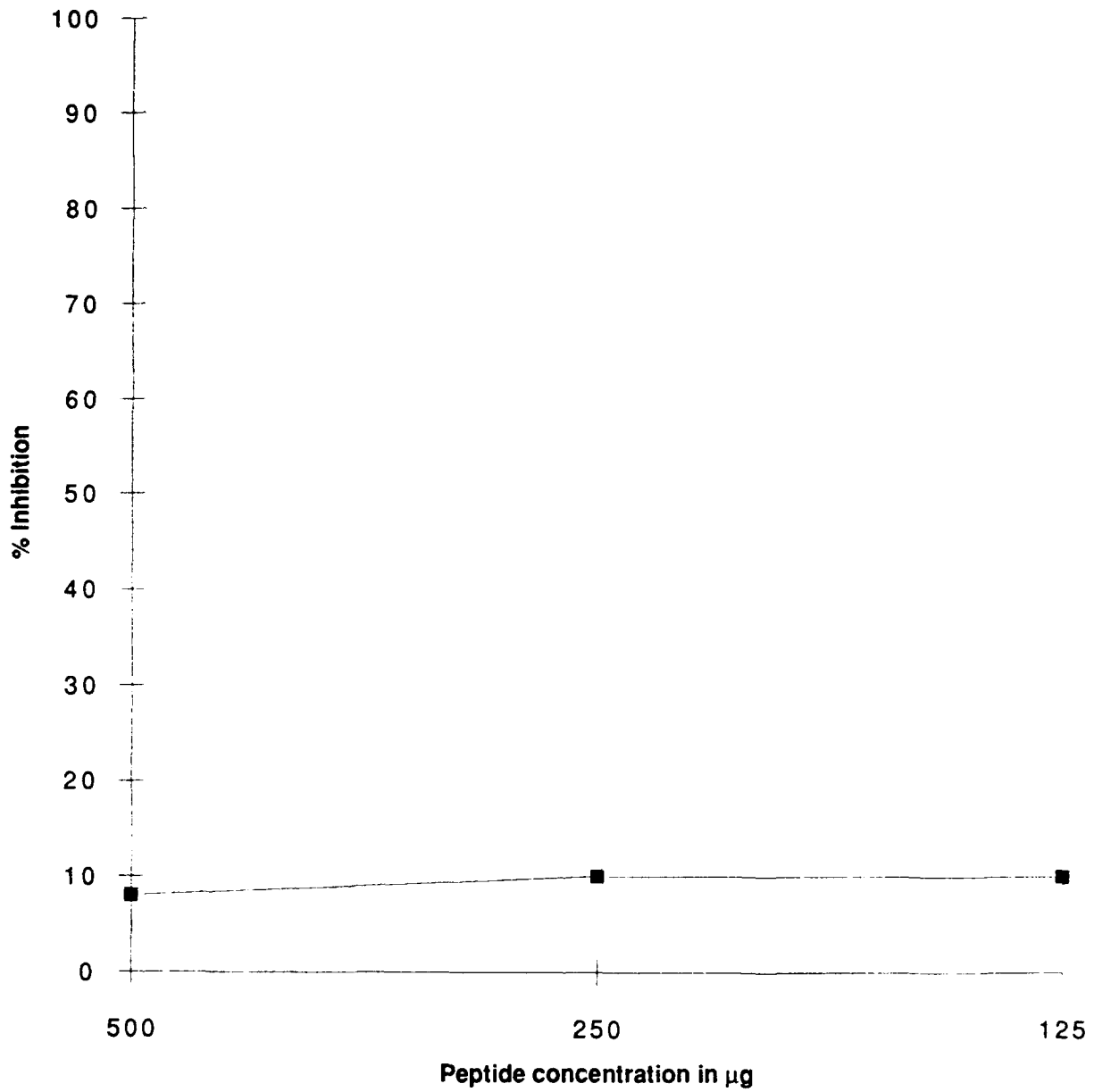


Figure 3

**Competitive inhibition for Receptor Binding
on 3T3 cells by PDGF B (104-116)**

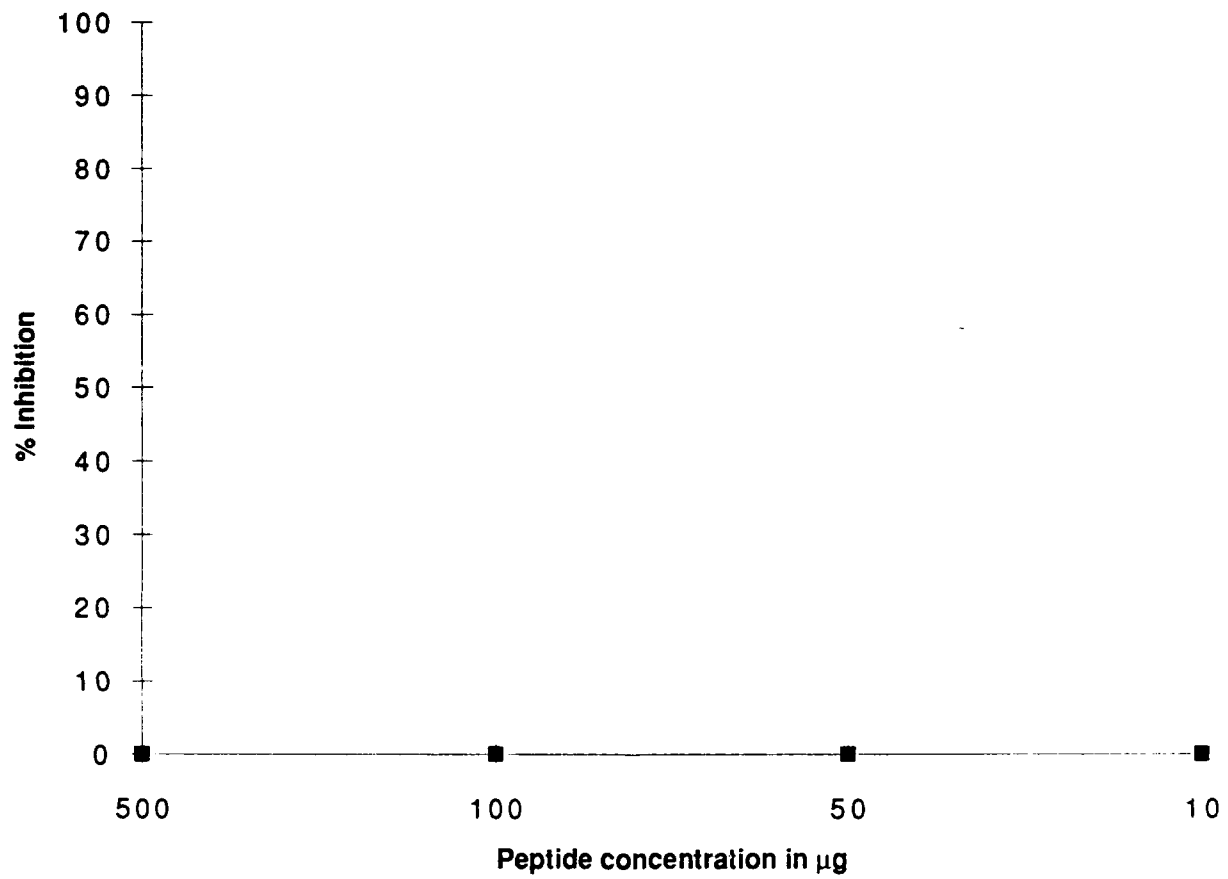
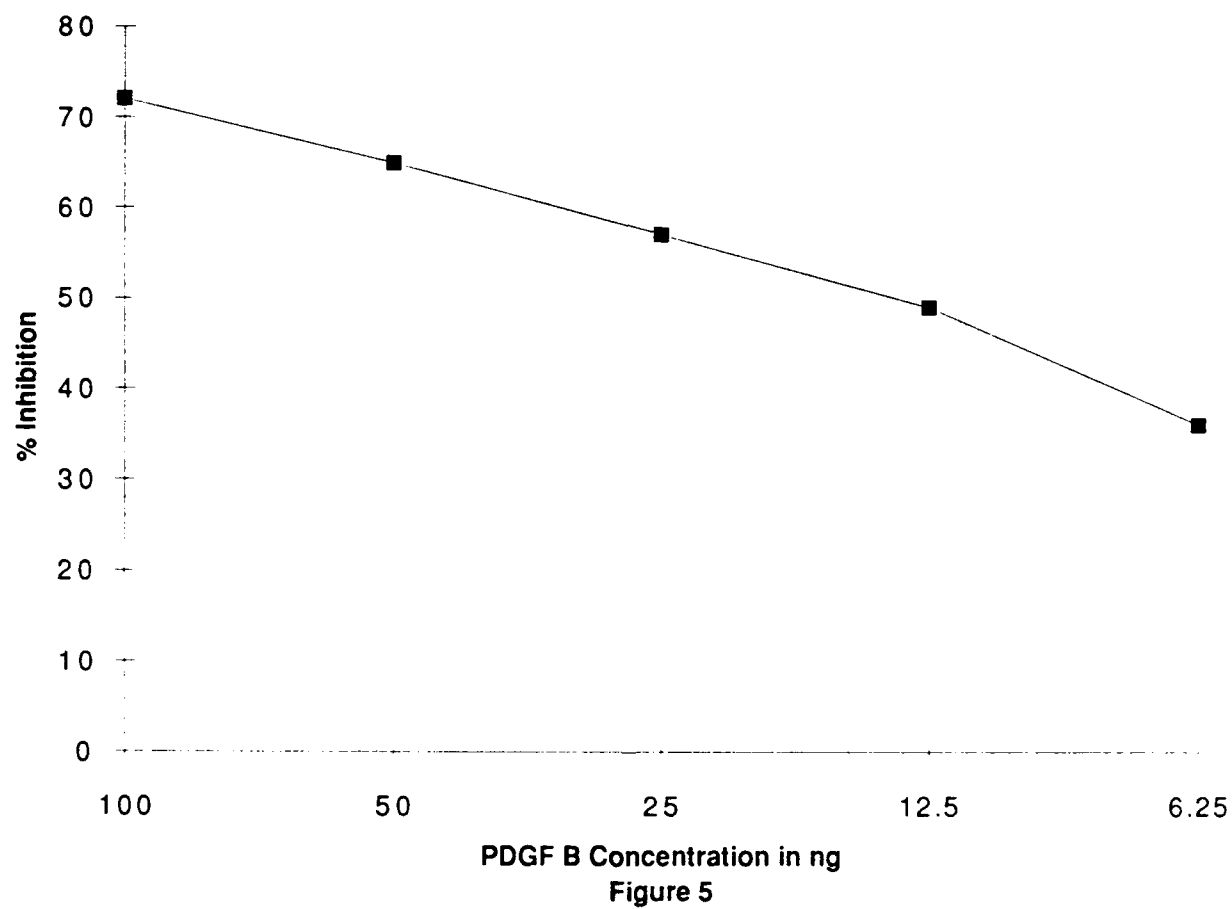


Figure 4

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF B**



MITOGENESIS ASSAY

The mitogenesis assay was performed on 3T3 cells by measuring the thymidine incorporation. So far we have completed the assay with PDGF. The results are shown in Figure 6. Assays with peptides are in progress.

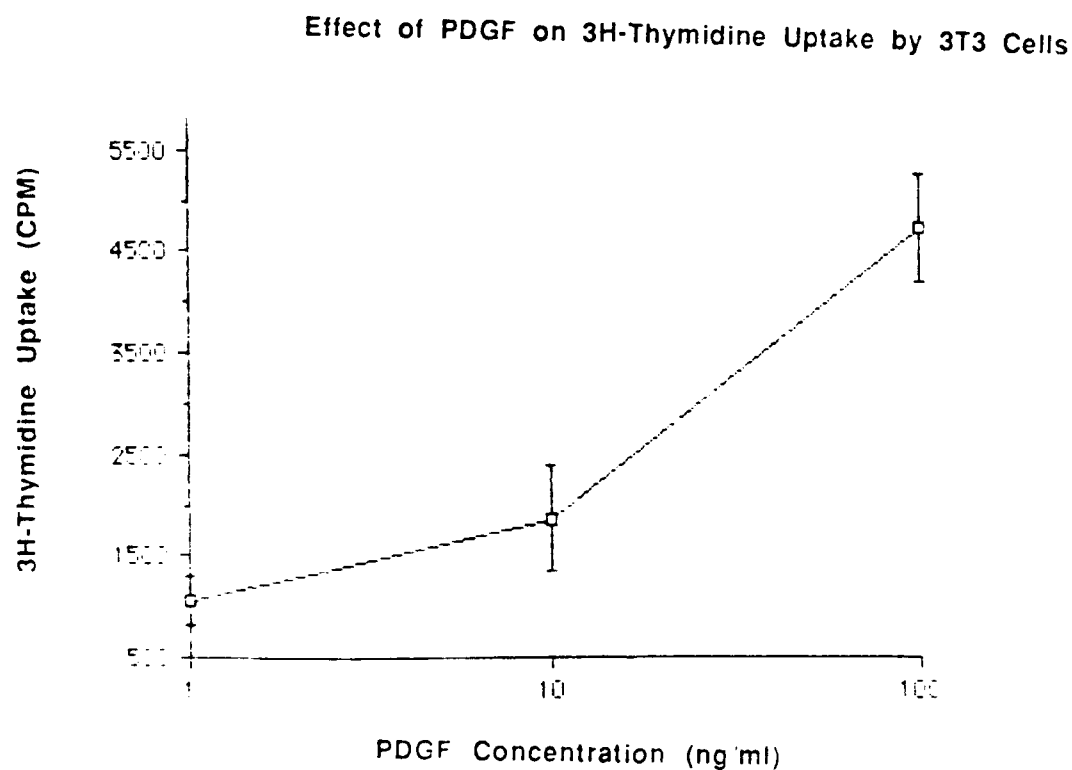


Figure 6

PLANS FOR NEXT QUARTER

During the next quarter we plan to synthesize more peptides; cell binding and mitogenesis assays will continue. Furthermore, we plan to optimize the conditions for chemotaxis assay.